Original Contribution

The Cold European Winter of 2005-2006 Assisted the Spread and Persistence of H5N1 Influenza Virus in Wild Birds

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Abstract: In January 2006, a major cold spell affected Europe, coinciding with an increase of H5N1 influenza virus detected in wild birds, mostly dead mute swans, starting along the River Danube and the Mediterranean coast line. Subsequently H5N1 detections in wild birds were concentrated in central and western parts of Europe, reaching a peak in mid February. We tested the hypothesis that the geographic distribution of these H5N1 infections was modulated by the long-term wintering line, the 0°C isotherm marking the limit beyond which areas are largely unsuitable for wintering waterfowl. Given the particularly cold 2005–2006 European winter, we also considered the satellite-derived contemporary frost conditions. This brought us to select the long-term maximum rather than the mean January 0°C isotherm as the best approximation for the 2005–2006 wintering line. Our analysis shows that H5N1 detection sites were closer to the wintering line than would be expected by chance, even when the geographic distribution of water bird wintering sites was accounted for. We argue that partial frost conditions in water bodies are conducive to bird congregation, and this may have enhanced H5N1 transmission and local spread. Because the environmental virus load also would build up in these hot spots, H5N1 virus may have readily persisted during the spring, at least in cooler areas. We conclude that H5N1 introduction, spread, and persistence in Europe may have been enhanced by the cold 2005–2006 winter.

Keywords: Avian influenza, waterfowl, outbreaks, seasonality, Europe

INTRODUCTION

The role of migratory birds as long-distance vectors of the highly pathogenic avian influenza (HPAI) H5N1 virus

(hereafter H5N1) has been much debated. The main arguments against an active role of migratory birds in the geographic spread of H5N1 are as follows. Immunosuppression resulting from intense flight activity would be expected to have a disproportional affect on H5N1 infected birds (Van Gils et al., 2007; Weber and Stilianakis, 2007). Very few H5N1 infected wild bird carcasses have been recovered along migratory routes. Poultry outbreak sites have not been observed to overlap closely and consistently

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with flyways (BirdLife International, 2006). Some countries in Asia frequented by migratory birds have never shown any H5N1 infection (Munoz et al., 2006). Even when large numbers of birds migrate each year through East Atlantic and East Asian-Australian flyways, the H5N1 virus has spread neither to the Americas nor to Australia (Gauthier-Clerc et al., 2007). Another, more specific argument against the role of wild, migratory birds is that the major die-off in wild birds recorded around Lake Qinghai in April 2005 took place several weeks after the migratory birds had arrived near the lake, suggesting that the virus was acquired at destination (Feare and Yasué, 2006; Weber and Stilianakis, 2008).

Conversely, the global pattern of H5N1 is generally consistent with the major bird migration routes (Kilpatrick et al., 2006; Si et al., 2009), and H5N1 virus spread has been found to broadly match bird migration in the Western Palearctic (Gilbert et al., 2006). Experimental infection of swans and geese has revealed that these birds may shed H5N1 virus before the onset of clinical signs (Brown et al., 2009). Wild *Anatidae*, with preexisting naturally acquired avian influenza virus-specific antibodies, may still excrete significant amount of virus asymptomatically (Kalthoff et al., 2008; Keawcharoen et al., 2008). In addition, healthy wild waterfowl have tested positive for highly pathogenic influenza viruses (Chen et al., 2006; Gaidet et al., 2008; Saad et al., 2007); an HPAI H5N2 infected white-faced whistling duck (*Dendrocygna viduata*), for example, could be tracked by satellite en route from Nigeria to Chad (Gaidet et al., 2008). Furthermore, recent studies of satellite-tracked, apparently healthy waterfowl have linked Lake Qinghai (China) to H5N1 infected sites in Mongolia (Prosser et al., 2009). In Europe, Kilpatrick et al. (2006) analyzed the roles of both migratory bird movements and poultry-related trade and transport and concluded that the spread of H5N1 to many of the European countries was most likely to have occurred through migratory birds.

Some of the characteristics of the H5N1 epidemic wave that first hit Europe in the autumn of 2005 implicated wild birds. The first detections of the disease in poultry in Turkey and Romania, in October 2005, were followed by H5N1 circulation in the wider Black Sea basin (Food and Agriculture Organization of the United Nations, 2006), with its numerous coastal wetlands and important wintering sites for many water bird species (Delany and Scott, 2006; Nankinov, 1996). The majority of the H5N1 cases in poultry have been recorded in the Danube delta, in the Crimea peninsula, and in Turkey (FAO, 2006). Here,

backyard poultry production is common and regular contact, or even cohabitation, with wild water birds occurs (Avram, 2008; Atkinson et al., 2006; Ward et al., 2008). In these ecological settings, hunting also may have a role in H5N1 transmission from wild birds to poultry, for example through disposal of offals in the backyard (Gill et al., 2006; Newman et al., 2008). Few H5N1 infections have been recorded in poultry in central and western parts of Europe, suggesting that perhaps here wild birds were the main actors (Si et al., 2009).

Several authors have remarked that the rapid westward expansion of the H5N1 virus recorded at the end of January 2006 was probably triggered by birds escaping the sudden frost conditions affecting the Black Sea basin (Gauthier-Clerc et al., 2007; Gilbert et al., 2006; Kilpatrick et al., 2006).

Although no direct evidence for the spread of the disease by wild bird has been collected, most H5N1-infected birds have been found dead (FAO, 2006). Wintering birds infected by H5N1 virus may succeed in leaving the areas affected by cold weather, and subsequently contribute to short-distance spread of the H5N1 virus (Weber and Stilianakis, 2007).

Extreme cold conditions are known to influence both long distance water bird migration and within-winter movements among different wintering sites (Baillie et al., 1986; Crick et al., 2006; Keller et al., 2009). Several environmental parameters, including low temperatures (specifically the minimum temperature in January) and the duration of the frost-free period, have been found to modulate the wintering distributions of bird species (Root, 1988). The wintering line, an eco-geographical boundary, is given by the 0°C isotherm of the mean temperature recorded in January (Alerstam, 1990; Cao et al., 2008; Gilissen et al., 2002). This line aims to define the limit beyond which areas are largely unsuitable for waterfowl to overwinter.

Our working hypothesis was that the overall distribution of H5N1 outbreaks in Europe was modulated by the wintering line and by the dynamic pattern of frost conditions recorded during the winter of 2005–2006, which affected the wintering behavior of water birds in the region. The wintering line, by definition, separates areas suitable for waterfowl to spend the winter from those that are unsuitable, based on the prevailing frost conditions.

If waterfowl did indeed play a major role in the 2005–2006 winter epidemic, we would have expected to observe none or few H5N1 detections to the north and east

of (beyond) the wintering line, where frost conditions prevented water fowl from spending the winter. To the south and west of the wintering line, however, cold conditions would be expected to be less extreme, wintering waterfowl more abundant, and H5N1 cases more likely.

METHODS

H5N1 Data

The geographic area selected comprises most of the Western Palearctic, which includes Europe to the west of the Ural mountain range, and Turkey. H5N1 outbreaks recorded in poultry and wild birds were extracted from the Food and Agriculture Organization (FAO) EMPRES-i database (FAO, 2006) for the period from October 2005, when the first case was recorded in Turkey, until mid-May 2006, which marked the end of the first major epidemic wave in Europe. Despite inevitable sampling biases, we believe that most H5N1 outbreaks in poultry came from Turkey whilst the bulk of H5N1 cases reported in wild birds originated from central and western parts of Europe. The geo-referenced data comprise 253 H5N1 outbreaks in poultry and 377 H5N1 cases in wild birds. Statistical analyses were performed only on H5N1 cases in wild birds. Cases may include multiple H5N1-infected birds. Bird species details were available for 261 wild bird cases, most of which were waterfowl. Sixty-seven percent of these were swans (*Cygnus*) and twenty-one percent dabbling and diving ducks (respectively, *Anatinae* and *Aythyinae*).

The epicurve of H5N1 cases recorded in wild birds was established using a locally weighted scatter plot (LOWESS), with a smoothing parameter ($\alpha = 0.1$) equivalent to a 9 days moving average.

Long-Term Temperature Data, Snow and Ice Cover, Wintering Line and Wintering Sites

Meteorological conditions affecting Europe during 2005 and 2006 were analyzed in terms of temperature, frost, and snow cover. Previous studies have highlighted that, in central and western parts of Europe, the winter of 2005–2006 was the coldest for more than a decade, characterized by mean surface air temperature anomalies ranging from 0°C to −2°C and by uncharacteristically deep snow, long snow-cover duration, and heavy intermittent snowfall events at low altitudes (Pinto et al., 2007; Scaife and Knight, 2008).

Information on freezing temperature conditions was derived from climatic raster grids, spatially interpolated to a spatial resolution of 1 km, available for the period 1950–1999 (Hijmans et al., 2005). We downloaded the long-term average of maximum temperatures in January (T_{max}) and transformed the floating point values into integers by truncating with $T_{max} = 0$. This raster was then vectorized to derive the wintering line.

Although the average January 0°C isotherm is considered to reflect the wintering line (Alerstam, 1990; Cao et al., 2008), others have reported that the January 0°C isotherm only broadly divides a warmer from a colder wintering zone (Kreuzbergh-Mukhina, 2006). Our choice to select T_{max} for the wintering line was based on the consideration that areas with $T_{max} \leq 0$°C do not experience any thawing conditions and are therefore highly likely to show ice formation, and/or the accumulation of a snow mantle. Such areas are likely to present environments that are not conducive to wintering waterfowl.

Information on snow cover was derived from daily snow and ice cover grids, at a spatial resolution of 4 km, downloaded from the National Snow and Ice Data Center (NOAA/NESDIS/OSDPD/SSD, 2006), for the period 1 October 2005 to 12 May 2006.

The need to monitor snow conditions until the spring was supported by the observation that, in central Europe, snow cover accumulation was recorded very late in the season (Pinto et al., 2007).

Because at a spatial resolution of 4 km ice cover only reveals very large lakes and wetlands, we considered ice and snow cover in conjunction (i.e., "snow–ice"). For this, we dichotomized each daily grid into presence/absence of snow–ice and reclassified pixels according to the number of days of snow–ice cover.

The combination of the T_{max} wintering line and the snow–ice distribution yielded two environmentally distinct areas. First is the area beyond (to the north and the east of) the wintering line, where January $T_{max} \leq 0$°C. Here, most water bodies are subjected to long periods of winter frost and the snow–ice cover lasts between 3 and 6 months. These areas offer very limited feeding possibilities for birds and would thus presumably be largely avoided or visited only briefly during winter.

The second area is that within (to the south and west of) the wintering line, where January $T_{max} > 0$°C. These areas are covered by snow–ice for periods of less than 3 months. Migratory waterfowl tend to overwinter here to minimize their distance from the summer breeding areas. In areas with

partial frost condition, water birds tend to congregate in and around any remaining, open water bodies rather than moving to more distant wintering sites, particularly given that the end of February or early March already marks the start of Anatidae spring migration (Arzel et al., 2006; Globig et al., 2009; Suter and Van Eerden, 1992).

The distribution of the main wintering sites for waterfowl in central and western parts of Europe was derived from Scott and Rose (1996), who report wintering sites that support at least 1% of the individuals in a population of a given waterfowl species. We incorporated 307 wintering sites that were considered important, in terms of population size, for the following species (listed in alphabetic order): common goldeneye (*Bucephala clangula*), common pochard (*Aythya ferina*), common teal (*Anas crecca*), Eurasian wigeon (*Anas penelope*), gadwall (*Anas strepera*), garganey (*Anas querquedula*), mallard (*Anas platyrhynchos*), mute swan (*Cygnus olor*), northern pintail (*Anas acuta*), northern shoveler (*Anas clypeata*), red-crested pochard (*Netta rufina*), and tufted duck (*Aythya fuligula*).

Statistical Analysis

The statistical analysis was designed to test the hypothesis that the distribution of H5N1 cases was concentrated around the January 0°C isotherm. In addition, we wanted to control for the possible confounding factor of the distribution of wintering sites. Indeed, if wintering sites were themselves distributed close to the January 0°C isotherm, the distribution of H5N1 cases observed in wild bird would follow the same pattern, which would reflect habitat preference rather than a relationship to the isotherm. To achieve this goal, we used a logistic regression model where the presence or absence of H5N1 cases (binary outcome) in a set of points was predicted as a function of two independent quantitative variable: the log-transformed distance to the January 0°C isotherm, and conversely, the log-transformed distance to the nearest wintering site. Using this model, a significant and negative effect of the distance to the January 0°C isotherm on the presence of H5N1 case would be indicative that these points are distributed closer to the isotherm than the negatives. The same applies to the distance to the nearest wintering site. A nice feature of this model is that it would allow quantifying simultaneously the relative influence of distance to the isotherm and to the wintering sites on the distribution of H5N1 cases. Two important points needed to be resolved to develop this model.

First, our data set was made of H5N1 cases only, which reflect presence, but we had no true absence points. This was addressed by distributing a high number of points considered as negatives through the land mass. Given the large extent of the analysis, we believed that sampling random points from the land gave a broader representation of the spectrum of areas potentially/temporarily used by waterfowl than would be obtained by sampling random points only from the main waterfowl wetlands recorded in Europe. The land from which our random points were selected includes not only all the 307 wetlands locations, but also all points potentially falling in other inland waters, such as large rivers and deltas, coastal systems, lakes, wetlands, rice fields, swamps, and artificial water bodies of smaller size. However, pooling a high number of points considered as negatives with the 377 H5N1 cases considered as positive was not possible because recent work demonstrated that logistic regression results could be biased when the positive-to-negative ratio was too low or too high (McPherson et al., 2004). To circumvent the problem, we had to use a number of negatives (randomly distributed points) matching the number of positives (H5N1 cases), and to bootstrap the analysis a high number of times so that the results would not depend on a particular set of points considered as negatives.

The second main point was the clustering in the distribution of H5N1 cases. The clumped distribution of the cases would likely result in spatial autocorrelation in the logistic regression model residuals, which has been shown to bias the estimate of the coefficient and the significance of individual terms of linear models (Keitt et al., 2002). We decided to use the autologistic regression approach proposed by Augustin et al. (1996). The autologistic regression model is similar to the logistic regression model, except that an auto-covariate term is added as variable to the set of independent variables. The auto-covariate term is a distance-weighted average of the dependent variable in a neighborhood (Dormann et al., 2007). The extent of this neighborhood needs first to be established by quantifying the spatial autocorrelation in the residuals of the standard logistic regression model.

More specifically, the analysis involved three main parts:

1) Distributing negatives

We generated 10,000 random locations on land distributed up to 664 km away from the wintering line, which was the maximum distance (d_{max}) found for H5N1 cases

from the wintering line. Those points were set to be at a distance higher than 1 km from any H5N1 case. This formed our set of points considered as negatives. The set of positives was made from the 377 H5N1 cases. For all points of both sets, we estimated the distance to the January 0°C isotherm, and the distance to the nearest wintering site.

2) Establishing the range of spatial autocorrelation

We sampled with replacement 377 of the set of 10,000 negatives, and pooled it with the set of positives to produce a data set containing 754 points. We performed a logistic regression model of the negative/positive status as dependent variable with the log-transformed distance to the January 0°C isotherm and the log-transformed distance to the nearest wintering site as independent variables. We then estimated the semivariance of the residuals of this model. This analysis was bootstrapped 500 times by changing each time the set of negatives sampled from the 10,000 set of negatives. This analysis established that 100 km was the distance beyond which no further spatial autocorrelation was detected and therefore a suitable distance to define the neighborhood of the auto-covariate term (see supplementary Figure).

3) Autologistic model

Again, we sampled with replacement 377 of the set of 10,000 negatives and pooled it with the set of positives to produce a data set with 754 points. We then performed an autologistic regression model of the negative/positive status as dependent variable with the log-transformed distance to the January 0°C isotherm, the log-transformed distance to the nearest wintering site, and the auto-covariate term as independent variables calculated using a range of 100 km. We recorded the significance of the Wald test (z statistic) for the individual terms of the model (distance to January 0°C isotherm, distance to wintering site, and auto-covariate term), and the χ^2 statistic of the log-likelihood ratio comparing the model with and without the distance to January 0°C isotherm. To assess the performance of the full model with both terms (distance to January 0°C isotherm and distance to wintering site) the area under curve (AUC) of the receiver operating characteristic (ROC) curve and proportion of deviance (Dc) explained by the model were recorded as indicators of goodness of fit (for those estimates, the auto-covariate term was not included in the model to avoid artificially inflating the goodness of fit estimate). The analysis was bootstrapped 500 times by changing each time the set of negatives sampled of the

10,000 set of negatives so that the outcomes would not depend on a particular set of negatives.

RESULTS

The full set of geo-referenced cases recorded in both poultry ($n = 253$) and wild birds ($n = 377$) from early October 2005 until mid-May 2006 are shown as a temporal plot in Fig. 1a, and geographically in Fig. 1b. Whilst most H5N1 detections in the Black Sea area occurred in poultry, records from central and western parts of Europe came mostly from infected wild birds. Figure 1 shows that virus circulation was initially confined to Turkey and the Black Sea basin. The end of January 2006 marked a sudden westward shift in H5N1 detection sites, mostly in wild birds.
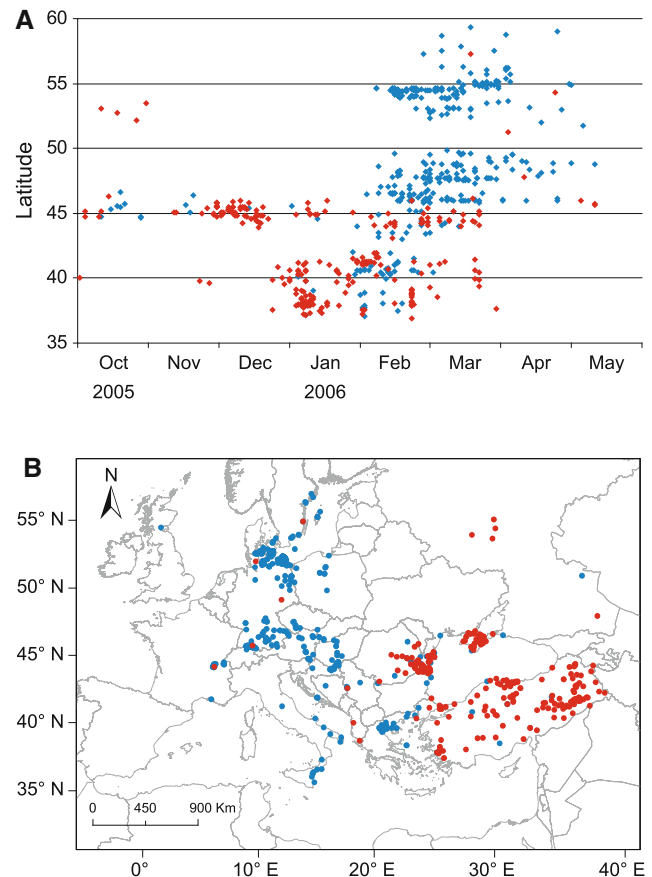

Figure 1. Temporal and spatial dynamics of H5N1 detections in poultry (*red dots*) and wild birds (*blue dots*), from October 2005 until mid-May 2006, are shown as a temporal plot (**a**) and geographically (**b**). The end of January 2006 marked a shift in H5N1 cases from poultry, primarily, to wild birds, which coincided with the sudden dispersal of H5N1 virus into central and western parts of Europe.
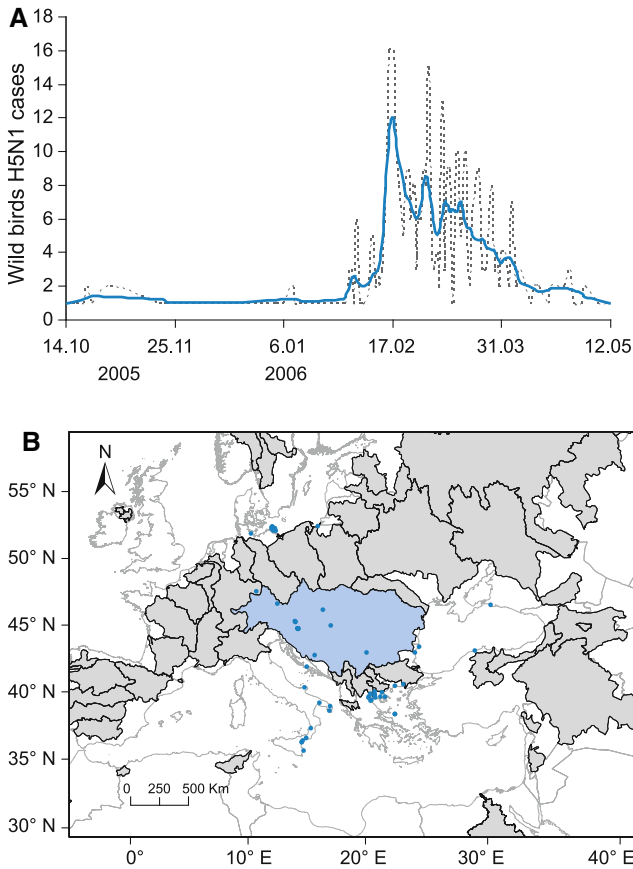
Figure 2. Temporal and spatial dynamics of H5N1 cases detected in wild birds. The time line in **a** runs from October 2005 until mid-May 2006 and is split into three phases; the initial prolonged period of establishment of H5N1 in eastern Europe, the sudden westwards dispersion at the end of January, followed by the persistence plus retraction phase. The epicurve (*blue line*) is based on a locally weighted scatter plot smoothing (LOWESS with $\alpha = 0.1$, equivalent to nine days moving average). The dispersion of H5N1 into central and western parts of Europe is further detailed in **b**, with the blue-coloured area depicting the Danube drainage system.

The abrupt westward H5N1 dispersion is shown in further detail in Fig. 2. Figure 2a depicts the epicurve for H5N1 cases in wild birds. After a prolonged period of only incidental detections, there was a sudden increase of H5N1 cases in wild birds starting at the end of January, reaching a peak around mid-February 2006, followed by a gradual persistence/retraction phase running until mid-May 2006. As shown in Fig. 2b, the sudden increase of H5N1 coincided with the geographic dispersion of H5N1 detections along the Mediterranean shoreline, in the Danube river system, and on the Baltic Sea coast of Germany. The occurrence of H5N1 in Germany on the Baltic Sea coast revealed a virus pertaining to a different sub-clade than the
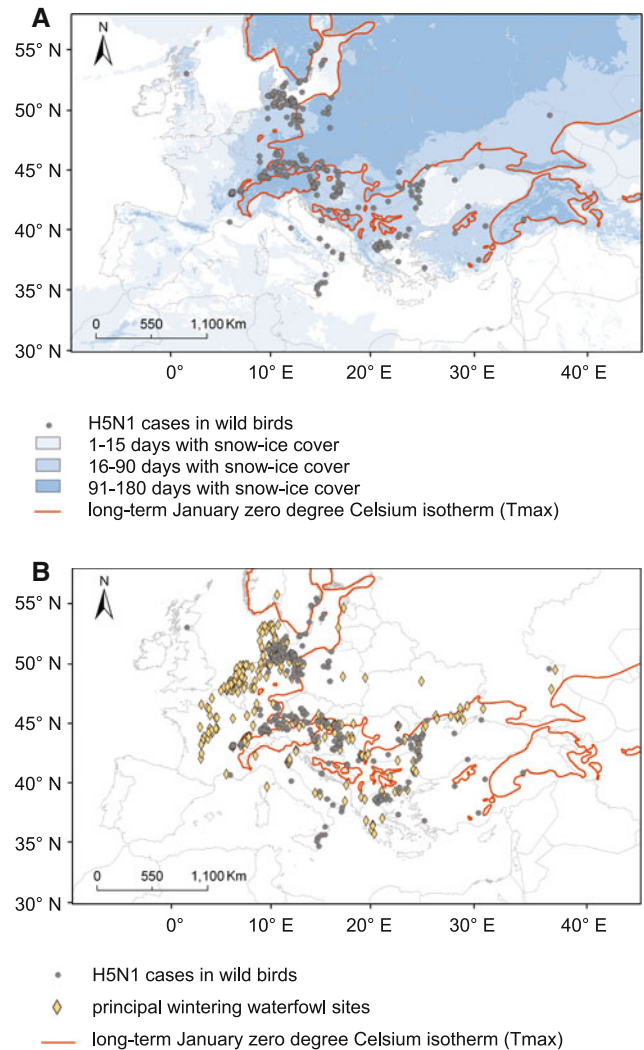

Figure 3. The geographic distribution of H5N1 cases in wild birds as recorded from October 2005 until mid-May 2006 (*grey dots*) together with the wintering line and the length of the period with snow-ice cover. The 78% of the H5N1 cases were detected in areas within (to the south and west of) the wintering line, given by the $T_{max} = 0°C$ isotherm (*red line*). Here, H5N1 transmission between birds and persistence in the environment, is presumably enhanced both by bird congregation in the remaining wintering sites and low temperatures preventing virus decay (**a**). The association of H5N1 cases in wild birds with the colder, higher latitude wintering grounds is shown in **b**.

virus encountered in the Danube system (Starick et al., 2008).

Figure 3a shows the geographic distribution of H5N1 cases in wild birds ($n = 377$) together with the T_{max} wintering line. Seventy-eight percent of H5N1 cases occurred within (to the south and west of) the T_{max} wintering line. A second observation was that the bulk of the H5N1 cases in wild birds were distributed among colder wintering grounds at higher latitudes (Fig. 3b).

Table 1. Results of the 500 Bootstrapped Autologistic Regression Model Runs

Goodness of fit of the model	1st. qu.			Median		3rd qu.
AUC	0.915			0.920		0.923
Dc	0.453			0.465		0.477

Variables included in the model	Wald statistic (z)			p value		
	1st qu.	Median	3rd qu.	1st qu.	Median	3rd qu.
Dist. to $T_{max} = 0$ (Log)	−3.28	−2.90	−2.41	0.0012	0.0036	0.013
Dist. to wintering site (Log)	−5.4	−4.93	−4.64	<0.001	<0.001	<0.001
Auto-covariate term	7.57	7.78	7.99	<0.001	<0.001	<0.001

Log-likelihood ratio chi-square test (LR)	1st qu.	Median	3rd qu.
p-value	<0.0001	0.00025	0.0014

AUC area under curve of the receiver operating character, *Dc* proportion of variance explained by the model, T_{max} long-term January 0°C isotherm given by T_{max}, *LR* log-likelihood ratio chi-square test comparing the model with and without the distance to the January 0°C isotherm ($T_{max} = 0$).
Statistics provided include goodness of fit metrics (AUC and Dc) and Wald z statistics and *p* values for the three variables in the model, together with the significance of the log-likelihood ratio chi-square test comparing the model with and without the distance to the January 0°C isotherm ($T_{max} = 0$).

The autologistic regression model showed that both the distance to T_{max} and to the wintering sites were significantly, inversely related to the presence of H5N1 cases in wild birds (Table 1). Spatial autocorrelation in the residuals of the logistic regression model was evident and was accounted for by an auto-covariate term up to a distance of 100 km (see supplementary Figure). This was confirmed in the autologistic regression model with an auto-covariate term that was found to be highly significant (Table 1). The autologistic model had good predictive power, with a median AUC value of 0.92, and a median proportion of explained deviance of 0.465. Finally, the added value of the distance to T_{max} was quantified by the log-likelihood ratio test upon removal of the variable from the model. This revealed the highly significant contribution of distance to T_{max} in explaining the distribution of H5N1 cases in wild birds.

DISCUSSION

Our hypothesis that the distribution of H5N1 outbreaks in Europe was modulated by the pattern of frost conditions was statistically tested. It was shown that the H5N1 cases occurred significantly closer to the wintering line than would be expected by chance. When the locations of the major waterfowl wintering sites were accounted for by the model, the proximity to the wintering line remained an important determinant of the observed spatial pattern. The implication of this is that low temperatures and frost conditions influenced the pattern of introduction, spread, and persistence of the H5N1 virus. This is commensurate with the speculations of other authors (Gauthier-Clerc et al., 2007; Gilbert et al., 2006; Kilpatrick et al., 2006) that the severe cold wave in Eastern Europe, which started in January 2006, might have pushed many wintering bird species away from their usual wintering grounds.

A major frost wave moved in from the east causing the Black Sea to freeze as far as 3–400 meters from its coast (Sabirovic et al., 2006). Shortly thereafter, several H5N1 infected mute swans were found dead along the Mediterranean shoreline (Fig. 2). Under extremely harsh weather conditions, mute swans (*Cygnus olor*) are known to evacuate areas that are completely frozen, forming large flocks in the process (Crick et al., 2006). One possible migration route for mute swans is through the Dardanelles along the Mediterranean coastline (Cramp and Simmons, 1977). However, the precise contribution of mute swans to the sudden dispersion of H5N1 remains elusive; mute swans may have acted mainly as sentinels for H5N1 virus circulation (Nagy et al., 2007). Other wild birds, including duck species, presumably also contributed to the introduction of H5N1 virus into central and western parts of Europe (Hars et al., 2008; Starick et al., 2008). That several bird species

played a role in vectoring H5N1 westward also is suggested by genetic evidence; multiple virus sub-clades were introduced in different countries (Rinder et al., 2007; Starick et al., 2008; Szeleczky et al., 2009).

Cold weather not only affects migration patterns and movements of waterfowl during the winter, but it ultimately shapes the distribution and local densities of waterfowl. The results of our model demonstrate that H5N1 outbreaks occurred closer to the wintering line than would be expected by chance. This finding should be interpreted on the basis of wintering waterbird ecology and behavior. Highly energy-demanding spring migration is undertaken by *Anatidae* when food resources en route and at the breeding grounds are still scarce (Arzel et al., 2006). Thus, keeping spring migration route short at the expense of wintering in colder areas close to the wintering line would ensure quick and less energy demanding trip to the breeding grounds, whilst wintering further away from wintering line and breeding grounds might be more beneficial strategy for individual survival, but not for reproductive success (Bregnballe et al., 2006; Steffen et al., 2008). Choice between these two strategies also is known to be species-, sex-, and age-dependent and often result in segregation of different groups at different latitudes (Choundhury and Black, 1991; Rustamov and Kovshar, 2007).

Close to the wintering line, frost conditions are expected to enhance waterfowl congregation and, with it, the probability of virus transmission and local spread. At a larger scale, completely frozen water bodies, which are uninhabitable to waterfowl, tend to result in increased concentrations of water birds in neighboring wetlands where open water is still available. A number of instances where H5N1 was detected in the winter of 2005–2006 at wintering sites where large number of water birds had congregated (Table supplementary material). The movement of water birds from neighboring water bodies has been suggested as an explanation for disease spread in some areas of central Europe. Wilking et al. (2009) reported that in Germany the initial outbreaks were invariably connected with the presence of water bodies, either seashore or freshwater lakes of various size. They suggested that local transmission through direct contact was facilitated by the clustered geographic distribution of the water bodies recorded on the Baltic coast and also at Lake Constance and in the Helme reservoir (Wilking et al., 2009). In other sites, however, such as the Dombes region in France, infections remained spatially more restricted, with several noninfected

ponds and a rather low mortality rate in wild birds (Hars et al., 2008).

At a local scale, frost conditions were found to enhance bird congregation when access to open water decreases and birds tend to crowd near remaining nonfrozen water (Spencer, 1982; Suter and Van Eerden, 1992). This was recorded in the Baltic Sea, where coastal shallows became covered with ice, interspersed with few areas of open water at which a diverse mixture of water bird species became concentrated (Globig et al., 2009).

It may be advantageous for water birds to stay in crowded water bodies rather than move elsewhere. Wintering migratory birds aim to increase fat storage to enhance their fitness in the forthcoming breeding season (Drent et al., 2006; Nilsson, 1979; Suter and Van Eerden, 1992; White and Bolen, 1984). Specialist feeders, such as diving ducks (Suter and Van Eerden, 1992) or mute swans, which are restricted to foraging in water less than 1 m deep (Scott and Rose, 1996), tend to remain faithful to the wintering location, even when this requires them to endure cold conditions (Suter and Van Eerden, 1992). Especially when a cold spell arrives late in the season, the energy cost involved in moving to more distant wintering sites may be high, whilst food resources at the alternative destination may already be depleted (Suter and Van Eerden, 1992). The level of aggregation in wintering ducks also has been shown to increase at the roosting sites because daily feeding flights are reduced in number in response to cold weather to conserve energy (Baldassare et al., 1986; Boos et al., 2002 Longcore and Gibbs, 1988; Owen and Cook, 1977; Ridgill and Fox, 1990). Low temperature not only plays a role in bird movements and aggregation but also might have enhanced H5N1 virus persistence in the environment.

Whilst presumably density-dependent, the precise mechanisms of direct transmission of H5N1 amongst water birds remain unclear. In the Dombes Region in France, densities of mute swans (*Cygnus olor*) and other water birds in infected ponds were sixfold and threefold, respectively, of those in uninfected ponds (Hars et al., 2008). Conversely, in other instances, relatively few infected birds were recorded in places with large populations of wintering birds (Gaidet et al., 2007; Globig et al., 2009; Hofmann et al., 2008; Nagy et al., 2007).

As the density of infected birds increases, the virus load of feces in water also will increase. H5N1 virus is well preserved under low temperature conditions (Brown et al., 2009). Environmental transmission appears to be important in sustaining periodic (every 2–4 years) influenza

outbreaks in North American wild waterfowl (Breban et al., 2009), and in the long-term cyclical preservation of virus under permafrost conditions (Lang et al., 2008).

It is noteworthy that H5N1 persisted within the Danube system, around the Alps and in the Baltic Sea drainage. These areas were subjected to persistent low temperatures during the extended winter of 2005–2006; southeast Germany and northeast Austria were still recording temperatures below zero and anomalies in snow depth as late as March (Pinto et al., 2007), and the Baltic Sea ice cover lasted until the end of April (Globig et al., 2009).

CONCLUSIONS

We conclude that the cold European winter of 2005–2006 may have assisted the introduction, spread, and persistence of H5N1 in wild birds. It follows that anomalous cold weather spells, similar to those recorded during the winter 2005–2006 (Pinto et al., 2007) would represent a warning signal to step up wild bird influenza surveillance in European wintering sites where partial frost conditions prevail.

DISCLAIMER

The views expressed in the publication are those of the authors and do not necessarily reflect the views of the Food and Agriculture Organization of the United Nations.

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